Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	4153	motoda.in. or yabuki.in. or kigawa.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L2	24385	"template DNA" or (template NEAR2 DNA)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L3	95880	"polymerase chain reaction" or PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L4	18631	(first or second or third) NEAR2 DNA	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L5	187021	primer or oligonucleotide	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L6	866	(first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L7	22503	(first or second or third) NEAR2 (primer or oligonucleotide)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L8	10644	(first or second or third) NEAR2 ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L9	442	"pmol/L"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L10	11	(motoda.in. or yabuki.in. or kigawa.in.) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L11	14383	("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L12	3967	(("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L13	465	(((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L14	315	((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L15	433	(((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L16	1	(((((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and "pmol/L"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L17	359	((((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L18	333956	multiplex$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L19	3363	multiplex$ WITH ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L20	48736	("polymerase chain reaction" or PCR) NEAR5 amplific$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L21	5783	(("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L22	2249	((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L23	1121	((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L24	1146	(((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) @pd<="2002"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L25	0	(((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and "PCR cloning"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L26	15171	(("polymerase chain reaction" or PCR) NEAR5 amplific$) and ("DNA template" or "template DNA" or "specific DNA fragments")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L27	8773	((("polymerase chain reaction" or PCR) NEAR5 amplific$) and ("DNA template" or "template DNA" or "specific DNA fragments")) and ((first or second or third) WITH primer)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L28	6418	nested WITH ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L29	202	(nested WITH ("polymerase chain reaction" or PCR)) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L30	678	(nested WITH ("polymerase chain reaction" or PCR)) SAME ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L31	322	((nested WITH ("polymerase chain reaction" or PCR)) SAME ((first or second or third) NEAR2 (primer or oligonucleotide))) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L32	327	(1st or 2nd or 3d) NEAR2 primer	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L33	11	((1st or 2nd or 3d) NEAR2 primer) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L34	1397	"two-step" NEAR2 PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L35	338	("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L36	66313	"affinity tag" or "maltose binding" or "cellulose binding" or "glutathione-s-transferase" or streptavidin or biotin or "his tag" or "histidine tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L37	244	("affinity tag" or "maltose binding" or "cellulose binding" or "glutathione-s-transferase" or streptavidin or biotin or "his tag" or "histidine tag") and (("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA)))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L38	4885	"6-His" or "histidine tag" or "6-his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L39	78	(("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA))) and ("6-His" or "histidine tag" or "6-his tag")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L40	537	"transcription template"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L41	5	"transcription template" and ("polymerase chain reaction" or PCR) and ((first or second or third) NEAR2 DNA) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L42	1	"native his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L43	2327	"protein synthesis" WITH "in vitro"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L44	2259	"protein synthesis" NEAR4 "in vitro"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L45	8	("protein synthesis" NEAR4 "in vitro") WITH cell-free	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L46	4153	motoda.in. or yabuki.in. or kigawa.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L47	24385	"template DNA" or (template NEAR2 DNA)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L48	95880	"polymerase chain reaction" or PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L49	18631	(first or second or third) NEAR2 DNA	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L50	187021	primer or oligonucleotide	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L51	866	(first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L52	22503	(first or second or third) NEAR2 (primer or oligonucleotide)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L53	10644	(first or second or third) NEAR2 ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L54	442	"pmol/L"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L55	14383	("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L56	3967	(("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L57	465	((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L58	315	((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L59	433	(((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L60	1	(((((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and "pmol/L"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L61	333956	multiplex$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L62	3363	multiplex$ WITH ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L63	48736	("polymerase chain reaction" or PCR) NEAR5 amplific$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L64	5783	(("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L65	2249	((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L66	1121	((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L67	1146	(((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) @pd<="2002"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L68	0	(((((("polymerase chain reaction" or PCR) NEAR5 amplific$) SAME ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and "PCR cloning"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L69	15171	(("polymerase chain reaction" or PCR) NEAR5 amplific$) and ("DNA template" or "template DNA" or "specific DNA fragments")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L70	8773	((("polymerase chain reaction" or PCR) NEAR5 amplific$) and ("DNA template" or "template DNA" or "specific DNA fragments")) and ((first or second or third) WITH primer)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L71	6418	nested WITH ("polymerase chain reaction" or PCR)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L72	678	(nested WITH ("polymerase chain reaction" or PCR)) SAME ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L73	327	(1st or 2nd or 3d) NEAR2 primer	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L74	1397	"two-step" NEAR2 PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L75	66313	"affinity tag" or "maltose binding" or "cellulose binding" or "glutathione-s-transferase" or streptavidin or biotin or "his tag" or "histidine tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L76	4885	"6-His" or "histidine tag" or "6-his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L77	537	"transcription template"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L78	1	"native his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L79	2327	"protein synthesis" WITH "in vitro"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L80	2259	"protein synthesis" NEAR4 "in vitro"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L81	11	(motoda.in. or yabuki.in. or kigawa.in.) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L82	11	((1st or 2nd or 3d) NEAR2 primer) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L83	78	(("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA))) and ("6-His" or "histidine tag" or "6-his tag")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L84	5	"transcription template" and ("polymerase chain reaction" or PCR) and ((first or second or third) NEAR2 DNA) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA))) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L85	8	("protein synthesis" NEAR4 "in vitro") WITH cell-free	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L86	202	(nested WITH ("polymerase chain reaction" or PCR)) SAME ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L87	322	((nested WITH ("polymerase chain reaction" or PCR)) SAME ((first or second or third) NEAR2 (primer or oligonucleotide))) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L88	244	("affinity tag" or "maltose binding" or "cellulose binding" or "glutathione-s-transferase" or streptavidin or biotin or "his tag" or "histidine tag") and (("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA)))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L89	359	((((("template DNA" or (template NEAR2 DNA)) SAME ("polymerase chain reaction" or PCR)) and ((first or second or third) NEAR2 DNA)) and ((first or second or third) NEAR2 ("template DNA" or (template NEAR2 DNA)))) and ((first or second or third) NEAR2 ("polymerase chain reaction" or PCR))) and (primer or oligonucleotide)) and ((first or second or third) NEAR2 (primer or oligonucleotide))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L90	338	("two-step" NEAR2 PCR) and ("template DNA" or (template NEAR2 DNA))	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L91	44982	endo.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32

L92	70	L91 and "cell free protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L93	5	L92 and "dilution batch"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L94	2209	template SAME (universal or mega)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L95	4737	"5' primer" SAME "3' primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L96	16665	promoter SAME terminator	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L97	10	L94 and L95 and L96	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L98	10	L97 and "PCR"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L99	0	"first sense primer" and "second sense primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L100	10	"first 5' primer" and "second 5' primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L101	231	"pcr generated" WITH template	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L102	0	L101 and "transcription temlate"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L103	0	L101 and "cell free protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32

L104	2626	"split primer" or "nested pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L105	24	L101 and L104	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L106	1051	"t7 promoter" WITH primer	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L107	43	L106 and L94	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L108	26301	gst or mbp or trxa or cbd or "his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L109	8	L107 and L108	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L110	41	mccarthy.in. and "cell free"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L111	24	L110 and PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L112	0	L111 and "overlapping sequences"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L113	7988	overlap WITH sequence	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L114	2	L111 and L113	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L115	207	barany.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L116	24385	"template DNA" or (template NEAR2 DNA)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L117	7	L115 and L116	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L118	0	L117 and "second pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L119	1	L115 and "second pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L120	1405	"multiplex PCR"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L121	133	L120 and "second pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L122	17	L121 and "t7 promoter"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L123	1405	"multiplex PCR"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L124	157	L123 and "cell free"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L125	31479	promoter and tag	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L126	114	L124 and L125	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L127	5496	"protein synthesis" and "cell free"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L128	72	L126 and L127	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L129	0	L128 and "second pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L130	2	"l64" and "second primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L131	1416848	overlapping sequence	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L132	4704	"overlapping sequence"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L133	66	L132 and L123	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L134	21	L133 and "second PCR"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L135	11	"5655563".pn. or "552302".pn. or "5492817".pn. or "5324637".pn. or "4966964".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L136	2530	"pcr amplified DNA"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L137	338	L136 and multiplex	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L138	0	L137 and "cell free protein synthesis"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L139	18	L137 and "protein synthesis"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32

L140	257	L137 and overlap$	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L141	15	"overlapping sequence" and L140	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L142	9201	"his" WITH tag	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L143	79	L140 and L142	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L144	30	L143 and ("t7 promoter" or "universal primer" or "universal promoter")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L145	1	kain.in. and "expression pcr"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L146	2	"5639595".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L147	1	"2000-264638"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L148	0	"2000-58404"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L149	1	"2000-058404"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L150	3	"2000-264638" or "2001-058404"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L151	3	"2000-261638" or "2001-058404"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32

L152	1	Lanar.in. and "cell free"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L153	57561	endo.in. or sawasaki.in. or ogasawara.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L154	10	L153 and "transcription template"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L155	5	"2000261638" or "2001058404"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L156	4	sorge.in. and "tagged molecules"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L157	0	L156 and "his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L158	4	L156 and "tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L159	0	L158 and promoter	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L160	6793	"his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L161	4353	L160 and "PCR amplification"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L162	473	L161 and multiplex	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L163	43	L162 and "second primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32

L164	452	L162 and "fusion protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L165	401	L164 and "affinity purification"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	OFF	2005/08/29 18:32
L166	349	L165 and ("tagged protein" or "tagged peptide")	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L167	1	L166 and "primer dimer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L168	17	L166 and "sense primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L169	344	L166 and "pcr primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L170	3026	"primer" SAME "his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L171	17	L169 and L170	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L172	10	"cell free protein expression" and "fusion protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L173	3026	primer SAME "his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L174	2838	L173 and "fusion protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L175	36	L174 and multiplex	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L176	66865	"affinity tag" or "maltose binding" or "cellulose binding" or "glutathione-s-transferase" or streptavidin or biotin or "his tag" or "histidine tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L177	1405	"multiplex pcr"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L178	731	L176 and L177	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L179	81332	"in vitro" or "cell free"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L180	263	L179 WITH ("protein expression" or "protein production")	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L181	3	L178 and L180	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L182	0	L181 and "his tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L183	0	L181 and "6 his"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32

L184	2647	Primer WITH (his or histidine)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L185	38	L184 and L177	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L186	7902	(multiplex or nested) WITH PCR	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L187	14615	(his or histidine) WITH tag	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L188	4214	primer same L187	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L189	385	L186 and L188	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L190	385	L189 and (sense primer)	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L191	262	L190 and "antisense primer"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32

L192	1	L191 and L180	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L193	244	"protein purification" and "6-his"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L194	7	"protein purification" SAME "6-his"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L195	2	"6207889".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L196	0	L195 and (6-his or histidine or "affinity tag")	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L197	0	"protein purification" SAME "6his tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L198	4493	"6 his tag" or "6-his tag" or "histidine tag" or "6xhis tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L199	195	L198 SAME ("protein purification" or "protein production")	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32

L200	2	"4683195".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L201	489	N-terminal WITH "his tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L202	423	c-terminal WITH "his tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L203	1	L201 and L177	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L204	12	L201 and L180	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L205	231	("affinity marker" or "affinity tag") same "his tag"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L206	45	(L201 or L202) and L205	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L207	12	L204 and primer	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32

L208	12	L207 and PcR	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2005/08/29 18:32
L209	39	rothschild.in. and "nascent protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L210	12	L209 and "c-terminal"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L211	10	L210 and "his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L212	1	"native his tag"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L213	8	tchaga.in. and "fusion protein"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L214	2	"5023171".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L215	5014	(amplification SAME overlap$) and primers	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L216	17479	single near2 reaction	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L217	884	L215 and L216	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L218	881	L217 and PCR	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L219	391	L218 and (sense and antisense)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

L220	280	L219 and tag	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L221	262	L220 and (cellulose or transferase or thioredoxin or streptavidin or histidine)	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L222	20	L221 and "universal primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L223	707	amplification same "universal primer"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L224	181	L223 and L216	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L225	49	L224 and nested	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L226	35	L225 and expression	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L227	52	("single tube" or "one-step") and L224	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L228	5200	motoda.in. or yabuki.in. or kigawa.in. or yokohama.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L229	30	L228 and "cell-free"	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32
L230	8	L229 and pcr	US-PGPUB; USPAT; EPO; JPO; DERWENT	OR	ON	2005/08/29 18:32

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☐ 1: Biotechniques. 1991 Mar;10(3):366-74. Related Articles, Links

Universal promoter for gene expression without cloning: expression-PCR.

Kain KC, Orlandi PA, Lanar DE.

Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

We present a rapid and simple system called expression-PCR (E-PCR) for in vitro synthesis of functional protein from genomic or plasmid DNA. A universal promoter was developed containing an untranslated leader sequence from alfalfa mosaic virus directly downstream from the T7 bacteriophage promoter. When this universal promoter is spliced to a DNA segment, it produces a suitable template for in vitro transcription and translation. The DNA to be expressed is first amplified by the PCR using a 5'-primer that incorporates an area homologous to the 3'-end of the universal promoter. The universal promoter and this DNA fragment are mixed and re-amplified in a reaction analogous to splicing by overlap extension, generating a recombinant DNA template that can be transcribed and translated in vitro without further processing. Unlike standard methods for in vitro transcription and translation, E-PCR is not dependent upon specialized transcription vectors, cloning, plasmid isolation and purification, or restriction enzyme sites. This approach has been used to synthesize and examine the biological activity of malaria proteins that are vaccine candidates for Plasmodium falciparum. E-PCR represents a significant improvement over current in vitro expression systems, most notably in its time savings, versatility of gene expression and its compatibility with rapid PCR-based site-directed mutagenesis procedures.

PMID: 2064773 [PubMed - indexed for MEDLINE]

☐ 1: J Immunol Methods. 1993 Jan 14;158(1):123-30. Related Articles, Links

Expression polymerase chain reaction for the in vitro synthesis and epitope mapping of autoantigen. Application to the human thyrotropin receptor.

Burch HB, **Nagy EV**, **Kain KC**, **Lanar DE**, **Carr FE**, **Wartofsky L**, **Burman KD**.

Department of Medicine, Walter Reed Army Medical Center, Washington, DC 20307-5001.

The clinical applicability of a newly described polymerase chain reaction directed protein expression system was assessed for the in vitro synthesis and partial epitope mapping of large radiolabeled human thyrotropin receptor (hTSH-R) protein segments. PCR amplification of targeted regions within the hTSH-R cDNA followed by in vitro transcription and translation permitted rapid synthesis of protein segments ranging in size from 18 to 62 kDa. Initial epitope mapping was directed at a 52 amino acid segment unique to the hTSH-R compared to otherwise homologous glycoprotein hormone receptors. Sera from Graves' disease patients known to have autoantibodies against the hTSH-R were used to immunoprecipitate two protein fragments differing only by the presence of the unique region in the larger fragment (E5) but not in the smaller fragment (E4). Dense precipitation bands were obtained using Graves' sera to immunoprecipitate E5 whereas little or no specific immunoprecipitation of E4 occurred. Normal sera gave only weak immunoprecipitation bands of E5. The technique provides significant advantages over conventional cloning methods and should have general applicability in the study of other protein targets of autoimmune disease.

PMID: 7679129 [PubMed - indexed for MEDLINE]

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Epitope mapping of protein antigens by expression-PCR (E-PCR).

Lanar DE, **Kain KC**, **Burch HB**.

Department of Immunology, Walter Reed Army Institute of Research, Washington, DC, USA.

PMID: 8959724 [PubMed - indexed for MEDLINE]

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☐ 1: PCR Methods Appl. 1994 Oct;4(2):S92-6. Related Articles, Links

Expression-PCR (E-PCR): overview and applications.

Lanar DE, Kain KC.

Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, USA.

Publication Types:
- Review
- Review, Tutorial

PMID: 7580880 [PubMed - indexed for MEDLINE]

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PubMed Central

☐ 1: Mol Gen Genet. 1984;195(1-2):300-7. Related Articles, Links

Structure and mitotic stability of minichromosomes originating in yeast cells transformed with tandem dimers of CEN11 plasmids.

Oertel W, Mayer M.

Large (10.5-13.5 kbp) circular minichromosomes containing the centromere of chromosome 11 (CEN11) and the MET14 gene of Saccharomyces cerevisiae in the YRp7 vector are considerably more stable during mitosis than smaller ones containing only the 1.6 kbp CEN11 SalI-fragment. Yeast transformants obtained with a tandem dimeric and thus dicentric form derived from this DNA varied in the mitotic stability of the TRP1 marker of the vector. The largest group of transformants contained minichromosomes which carried deletions located quite specifically at one of the two centromeres in the dimer, eliminating its function in mitosis. This group included also some minichromosomes which had been modified by intramolecular tandem amplification of the subunit carrying the deletion without losing the centromere within the unmodified subunit. The second major group carried minichromosomes which had been monomerized. Monomerized minichromosomes showed the relative low degree of mitotic stability typical for the original minichromosomes containing the 1.6 kbp CEN11 SalI-fragment. Increasing numbers of additional subunits carrying the TRP1-ARS1 sequences but lacking additional centromeres improved the mitotic stability considerably.

PMID: 6092856 [PubMed - indexed for MEDLINE]

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L3       421900 S "POLMERASE CHAIN REACTION" OR PCR
L4        78315 S EXPRESSION (S) L3
L5          706 S (TWO-STEP OR 2-STEP) (2A) PCR
L6           20 S (1ST OR 2D OR 2ND OR 3D) (2A) PRIMER
L7        11328 S NESTED (2A) PCR
L8       287986 S PROTEIN (S) SYNTHES?
L9       684655 S "POLYMERASE CHAIN REACTION" OR PCR
L10        1347 S "AFFINITY TAG" OR "PROTEIN TAG" OR 6-HIS OR "6-HIS TAG"
L11         915 S (FIRST OR SECOND OR THIRD) (3A) OLIGONUCLEOT?
L12         440 S RIKEN?
L13        5274 S EXPRESSION (3W) (PCR OR "POLYMERASE CHAIN REACTION" OR AMPLIF
L14        7482 S KAIN?/AU OR LANAR?/AU
L15          15 S L14 AND L13
L16          10 DUP REM L15 (5 DUPLICATES REMOVED)
L17           6 S L1 AND L2 AND L3
L18           4 DUP REM L17 (2 DUPLICATES REMOVED)
L19           9 S L12 AND L1
L20           6 DUP REM L19 (3 DUPLICATES REMOVED)
L21           3 S L5 AND L13
L22           1 DUP REM L21 (2 DUPLICATES REMOVED)
L23           0 S L10 AND L11
L24          12 S L13 AND L2 AND L4
L25           8 DUP REM L24 (4 DUPLICATES REMOVED)
L26          76 S L2 AND L9 AND L8
L27          25 S L26 AND PRIMERS
L28          18 DUP REM L27 (7 DUPLICATES REMOVED)
L29          15 S L28 NOT PY>=2004
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L18 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2005401512 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16061975
TITLE: Detection of HTLV-1 gene on cytologic smear slides.
AUTHOR: Kashima Kenji; Daa Tsutomu; **Yokoyama Shigeo**
CORPORATE SOURCE: Department of Pathology, Oita University, Oita, Japan.
SOURCE: Methods in molecular biology (Clifton, N.J.), (2005) 304 183-9.
Journal code: 9214969. ISSN: 1064-3745.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050803
Last Updated on STN: 20050804

AB In this chapter we describe a method for the detection of human T-cell leukemia virus type 1 (HTLV-1) genes in cytologic smears by polymerase chain reaction (**PCR**). First, already-stained and covered slides should be immersed in xylene for removal of cover slips. After passage through a descending ethanol series, slides are ready for DNA extraction. If the neoplastic cells on slides are mixed with nonneoplastic lymphocytes, cells of interest are isolated by microdissection. Two easy methods to dissect the samples using hydrophobic and hydrophilic mounting media are detailed. Second, microdissected cells are collected in microtubes and digested with proteinase K. The cells that did not undergo the microdissection are digested and dissolve in the proteinase K solution on the slides. Last, the **template DNA** is extracted from the solution and provided to **PCR**. We use two sets of primers for detection of HTLV-1 genes, and the products of amplification by **PCR** that correspond to the pX and tax regions are expected to be 127 and 159 base pairs long, respectively. Although this method does not provide proof of the monoclonal integration of HTLV-1 genes, it can be applied when adult T-cell leukemia/lymphoma is suspected cytologically but fresh samples for Southern blotting are unavailable.

L18 ANSWER 2 OF 4 EMBASE on STN
ACCESSION NUMBER: 2002262852 EMBASE
TITLE: Detection of the HTLV-I gene on cytologic smear slides.
AUTHOR: Kashima K.; Nagahama J.; Sato K.; Tanamachi H.; Gamachi A.; Daa T.; Nakayama I.; **Yokoyama S.**
CORPORATE SOURCE: Dr. K. Kashima, Department of Pathology, Oita Medical University Hospital, Hasamamachi, Oita 879-5593, Japan. kkashima@oita-med.ac.jp
SOURCE: Acta Cytologica, (2002) Vol. 46, No. 4, pp. 709-712.
Refs: 7
ISSN: 0001-5547 CODEN: ACYTAN
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
005 General Pathology and Pathological Anatomy
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20020808
Last Updated on STN: 20020808

AB OBJECTIVE: To apply the polymerase chain reaction (**PCR**) for detection of the HTLV-I gene from cytologic smear slides. STUDY DESIGN: Samples were from seven cases of serum anti-ATL antibody (ATLA)-positive T-cell lymphoma and three from ATLA-negative T-cell lymphoma. Six of the seven ATLA-positive cases were confirmed to be ATLL by Southern blotting. From the seventh case afresh sample for blotting could not be obtained. DNA was extracted from the cytologic smear slides of all 10 cases; they had been stained with Papanicolaou or May-Giemsa stain, digested with proteinase K and precipitated with phenol and ethanol. The target sequence in the pX region of the HTLV-I gene was amplified by **PCR**. RESULTS: All seven ATLA-positive cases, including one that had not yet

been confirmed by Southern blotting, showed a single band, as predicted, while the three ATLA-negative cases showed no band. CONCLUSION: If cytologic smear slides are available but a fresh sample is not, the **PCR** method should provide evidence that the virus is present since in our study sufficient DNA templates were successfully extracted from the stained cytologic smear slides for detection of the virus.

L18 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 95330966 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7606922
TITLE: Improved simple generation of GTG-band specific painting probes.
AUTHOR: **Yokoyama Y**; Sakuragawa N
CORPORATE SOURCE: Department of Chromosome Analysis, Center for Molecular Genetics, Hachioji, Japan.
SOURCE: Cytogenetics and cell genetics, (1995) 71 (1) 32-6.
Journal code: 0367735. ISSN: 0301-0171.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950828
Last Updated on STN: 20000303
Entered Medline: 19950816

AB We developed an improved, simple method of generating chromosome-region-specific probes from only a few microdissected chromosomes. One to five dissected fragments from a defined chromosomal region were processed with a PEG/proteinase K cycling deproteinization step and directly amplified with a two-step amplification system using a degenerate oligonucleotide primed shuttle polymerase chain reaction (DOP-Shuttle-**PCR**). This modified method offered three advantages over previously reported methods: relaxation of the highly condensed chromosomal **DNA**, reduction of the risk of endogenous and exogenous contamination, and high efficiency amplification of **template DNA**. High intensity in the fluorescence in situ hybridization (FISH) signals from normal metaphase chromosomes, as well as regional specificity of these probes, corresponding to regions on R-banded chromosomes, were observed.

L18 ANSWER 4 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1994:59551 BIOSIS
DOCUMENT NUMBER: PREV199497072551
TITLE: Detection of densonucleosis virus in the silkworm, Bombyx mori, from fecal specimens by a polymerase chain reaction.
AUTHOR(S): Abe, Hiroaki [Reprint author]; Shimada, Toru; Kobayashi, Kaori [Reprint author]; Maeda, Susumu; **Yokoyama, Takeshi** [Reprint author]; Oshiki, Toshikazu [Reprint author]; Kobayashi, Masahiko
CORPORATE SOURCE: Fac. Agric., Tokyo Univ. Agric. Technol., Fuchu, Tokyo 183, Japan
SOURCE: Journal of Sericultural Science of Japan, (1993) Vol. 62, No. 5, pp. 376-381.
CODEN: NISZAQ. ISSN: 0037-2455.
DOCUMENT TYPE: Article
LANGUAGE: Japanese
ENTRY DATE: Entered STN: 9 Feb 1994
Last Updated on STN: 9 Feb 1994

AB Feces from silkworm larvae, Bombyx mori, infected with Bombyx densonucleosis virus type-1 (DNV-1) and type-2 (DNV-2) contain viruses particles. A polymerase chain reaction (**PCR**) was used to detect DNV infected silkworm larvae without killing. Newly-ecdysed 4th instar larvae of susceptible and nonsusceptible (completely resistant) strains were fed DNVs 24 hours prior to the collection of feces. Feces were collected daily and **DNA** extracted from a single specimen of fecal matter was used as a **template** for **PCR**. In the susceptible strains, DNVs were detected in all fecal specimens beginning the day after inoculation to the day of viral death. In the nonsusceptible strains, DNVs were detected only in fecal matter collected

the day after inoculation and were not detected on and after the 2nd day. Based on these studies, we were able to diagnose rapidly both DNV-1 and DNV-2 infection using **PCR** to detect DNVs in fecal matter.

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L25 ANSWER 1 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 2004382537 EMBASE
TITLE: Cloning vectors for **expression-PCR** products.
AUTHOR: Betton J.-M.
CORPORATE SOURCE: J.-M. Betton, U. Repliement/Modelisation Proteines, Institut Pasteur, CNRS-URA2185, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France. jmbetton@pasteur.fr
SOURCE: BioTechniques, (2004) Vol. 37, No. 3, pp. 346-347.
Refs: 12
ISSN: 0736-6205 CODEN: BTNQDO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
ENTRY DATE: Entered STN: 20040924
Last Updated on STN: 20040924
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L25 ANSWER 2 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
ACCESSION NUMBER: 2004204552 EMBASE
TITLE: Increased sample capacity for genotyping and **expression** profiling by kinetic **polymerase chain reaction**.
AUTHOR: Watson R.M.; Griaznova O.I.; Long C.M.; Holland M.J.
CORPORATE SOURCE: M.J. Holland, Department of Biological Chemistry, School of Medicine, University of California, One Shields Avenue, Davis, CA 95616, United States. mjholland@ucdavis.edu
SOURCE: Analytical Biochemistry, (1 Jun 2004) Vol. 329, No. 1, pp. 58-67.
Refs: 20
ISSN: 0003-2697 CODEN: ANBCA2
PUBLISHER IDENT.: S 0003-2697(04)00166-6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040628
Last Updated on STN: 20040628
AB We fabricated and evaluated high-throughput kinetic thermal cyclers with 768-reaction capacity for kinetic polymerase chain reaction (kPCR)-based genotyping and kinetic reverse transcription (kRT)-PCR-based transcript quantitation. The system uses dye-based detection with ethidium bromide and a single DNA polymerase-based PCR or RT-PCR assay. Allele-specific detection of the two most common hereditary hemochromotosis mutant alleles, C282Y and H63D, was reliably measured by kPCR using human DNA templates as low as 10 genome equivalents per assay. Transcript profiling was performed for 16 yeast transcripts ranging in intracellular abundance over four orders of magnitude. Standard deviations of the PCR cycle threshold values determined from multiple kRT-PCR assays in three different instruments ranged from 0.11 to 0.97 PCR cycles and were reproducible, transcript specific, and instrument independent. The effects of the sin3, gal11, and snf2 knockout mutations on **expression** of 385 yeast genes were evaluated by kRT-**PCR** and compared to published values determined by high-density oligonucleotide array and/or microarray analysis for snf2 and sin3. The 768-reaction kinetic thermalcyclers, each with a capacity for more than a half million assays per year, are well suited to genomics applications such as single nucleotide polymorphism/disease association studies and genomewide transcription profiling where high sensitivity and accuracy are required. .COPYRGT. 2004 Elsevier Inc. All rights reserved.

L25 ANSWER 3 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:381226 BIOSIS

DOCUMENT NUMBER: PREV200000381226
TITLE: Restriction primers as short as 6-mers for PCR amplification of bacterial and plant genomic DNA and plant viral RNA.
AUTHOR(S): Ryu, Ki Hyun [Reprint author]; Choi, Sun Hee; Lee, Jong Suk
CORPORATE SOURCE: Plant Virus GenBank, Department of Horticultural Science, College of Natural Science, Seoul Women's University, Seoul, 139-774, South Korea
SOURCE: Molecular Biotechnology, (Jan., 2000) Vol. 14, No. 1, pp. 1-3. print.
ISSN: 1073-6085.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Sep 2000
Last Updated on STN: 8 Jan 2002

AB Amplification of DNA or RNA sequences using the polymerase chain reaction (PCR) or reverse transcriptase PCR (RT-PCR) requires primers of an appropriate length to be designed. Two hexamer restriction primers, denoted as E101 and H301, which correspond to sequences of EcoRI and HindIII recognition sites, respectively, were selected and used as primers in PCR and RT-PCR. We first applied the restriction primers to the plasmid DNA and bacterial (Pseudomonas) and plant (Cymbidium) genomic DNAs. We observed positive DNA amplifications with the recombinant plasmid DNA and bacterial and plant genomic DNAs. Purified viral RNA was used for **template** in the RT-PCR with the primers and successful **DNA** amplification was obtained. These results suggest that the 6-mer restriction primers can be useful for new applications in PCR.

L25 ANSWER 4 OF 8 EMBASE
on STN
ACCESSION NUMBER: 1999221267 EMBASE
TITLE: Efficient coupled transcription/ translation from PCR template by a hollow-fiber membrane bioreactor.
AUTHOR: Nakano H.; Shinbata T.; Okumura R.; Sekiguchi S.; Fujishiro M.; Yamane T.
CORPORATE SOURCE: T. Yamane, Lab. of Molecular Biotechnology, Dept. of Biol. Mechanisms/Functions, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan.
yamanetu@agr.nagoya.u.ac.jp
SOURCE: Biotechnology and Bioengineering, (20 Jul 1999) Vol. 64, No. 2, pp. 194-199.
Refs: 14
ISSN: 0006-3592 CODEN: BIBIAU
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19990708
Last Updated on STN: 19990708

AB A novel bioreactor using a hollow-fiber membrane was developed for the coupled transcription/ translation system using T7 RNA polymerase and Escherichia coil S30 extract. The large surface area per the reaction volume of the reactor assured rapid mass transfers of substrates into the reaction mixture and of wastes out from it across the membrane by their molecular diffusion. The flux was large enough to maintain nucleotide concentrations for more than 3 h, which increased the protein synthesis greatly. In addition, the T7 terminator sequence downstream from the reporter genes was found to increase the synthesized protein significantly, especially when the product of polymerase chain reaction (PCR) was used as a template. Implementation of this finding and use of the bioreactor developed multiplied the productivity of protein by the in vitro direct **expression** from **PCR** template.

L25 ANSWER 5 OF 8 EMBASE
on STN DUPLICATE 1

ACCESSION NUMBER: 1999030522 EMBASE
TITLE: Semiquantitative determination of human cytokine mRNA **expression** using TaqMan RT-**PCR**.
AUTHOR: Lang R.; Heeg K.
CORPORATE SOURCE: R. Lang, Med. Microbiol./Immunol./Hyg. Inst., Technical University of Munich, Trogerstr. 32, 81675 Munich, Germany
SOURCE: Inflammopharmacology, (1998) Vol. 6, No. 4, pp. 297-309. Refs: 17
ISSN: 0925-4692 CODEN: IAOAES
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19990304
Last Updated on STN: 19990304

AB To establish an easy, fast and reliable RT-**PCR** for the analysis of human cytokine **expression**, we made use of the recently developed technique of TaqMan **PCR**. This technique is based on the cleavage of fluorochrome-labelled internal oligodeoxynucleotide probes by the 5' → 3' nuclease activity of Taq DNA polymerase. Measurement of fluorescence intensity during each cycle of the PCR reaction with a Sequence Detection System allows the determination of a threshold cycle at which an increase in fluorescence intensity is first detectable. From these values, a starting amount of **template DNA** can be calculated. Here, we established specific primers and corresponding internal, fluorogenic probes for the human cytokines tumour necrosis factor-α (TNF- α), interleukin-1β (IL-1β) and interferon-γ (IFN-γ), and for the constant region of the T-cell receptor β chain (TCRβ) and the housekeeping gene, glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) for normalization of mRNA expression levels. Titrations of the cDNA input showed a strict inverse correlation between the threshold cycles obtained and the starting amount of template. This in turn allowed the generation of a standard curve, and thus quantification of mRNA abundance in cDNA samples. Evaluation of the method using cDNAs from peripheral blood mononuclear cells (PBMC) stimulated with lipopolysaccharide (LPS) or phytohaemagglutinin (PHA) showed basal expression of TNF-α and IL-1β in untreated PBMC while IFN-γ was not detectable or only weakly expressed. After stimulation with LPS, a strong induction of IL- 1β and TNF-α was measured, while IFN-γ was induced to a lesser extent. PHA treatment, in contrast, led to an induction of all three cytokines with IFN- γ being the most prominent. The method has a large dynamic range, requires no post-PCR processing and gives reliable results.

L25 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 97443354 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9298215
TITLE: Quantification of transcript-to-template ratios as a measure of gene **expression** using RT-**PCR**
AUTHOR: Harting I; Wiesner R J
CORPORATE SOURCE: University of Heidelberg, Germany.
SOURCE: BioTechniques, (1997 Sep) 23 (3) 450-5. Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Report; (TECHNICAL REPORT)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971029

AB A new protocol was established for the quantitative analysis of gene expression in small muscle biopsies. Reverse transcription-PCR was performed with a preparation of total nucleic acids (**DNA**+RNA), amplifying the sequences of interest (targets; mitochondrial transcripts:

12S rRNA, cytochrome-c-oxidase [COX I] mRNA) together with an endogenous, non-transcribed reference sequence (**template**: D-loop region of mtDNA). Synthesis of PCR products at consecutive cycles within the exponential phase was quantified by measuring incorporation of radioactivity. Product accumulation was determined by regression analysis of these data. Gene expression could then be quantified as a ratio of target transcripts to reference DNA. The results revealed a ratio of 12S rRNA:mtDNA and COX I mRNA:mtDNA of 14 and 2, respectively, or a ratio of 12 S rRNA:COX I mRNA of 7 in human left ventricle and are in good agreement with previously published values for rat liver and muscle. In addition to the investigation of mitochondrial gene expression in the steady state and during mitochondrial proliferation, this newly developed method will easily be applicable to expression analysis of any nuclear gene using an intron sequence as endogenous reference.

L25 ANSWER 7 OF 8 EMBASE
on STN

ACCESSION NUMBER: 1998153407 EMBASE
TITLE: In vitro **expression** of **PCR** fragments: A convenient tool for generating purified recombinant proteins in analytical amounts.
AUTHOR: Nechansky A.; Ruf C.; Kricek F.
CORPORATE SOURCE: Dr. F. Kricek, Novartis Forschungs Institut GmbH, Brunner Strasse 59, A-1235 Vienna, Austria. FRANZ.KRICEK@pharma.novartis.com
SOURCE: Methods in Molecular and Cellular Biology, (1995) Vol. 6, No. 2, pp. 94-103.
Refs: 8
ISSN: 0898-7750 CODEN: MMCBEV
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19980604
Last Updated on STN: 19980604

AB A method is presented in which an in vitro gene expression system for linear DNA templates based on bacterial extracts was directly coupled to a protein purification method. This method allows the production of purified, biologically active, recombinant proteins directly from polymerase-chain- reaction-amplified DNA containing all regulatory elements necessary for transcription and translation in a one-tube reaction within a few hours. The purified material can be directly used in biological testing. Because in vitro transcription or translation is carried out in bacterial extracts, subsequent large-scale production of the desired recombinant protein can be performed in Escherichia coli by using the same construct as for the in vitro expression, thus avoiding laborious and time-consuming subcloning. Furthermore, by using this system, host cell transformation and growth, in vivo gene expression, and protein purification procedures can be avoided for many applications.

L25 ANSWER 8 OF 8 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 91291372 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2064773
TITLE: Universal promoter for gene **expression** without cloning: **expression-PCR**.
AUTHOR: Kain K C; Orlandi P A; Lanar D E
CORPORATE SOURCE: Department of Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.
SOURCE: BioTechniques, (1991 Mar) 10 (3) 366-74.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910901
Last Updated on STN: 19910901

Entered Medline: 19910814

AB We present a rapid and simple system called **expression-PCR** (E-**PCR**) for in vitro synthesis of functional protein from genomic or plasmid DNA. A universal promoter was developed containing an untranslated leader sequence from alfalfa mosaic virus directly downstream from the T7 bacteriophage promoter. When this universal promoter is spliced to a **DNA** segment, it produces a suitable **template** for in vitro transcription and translation. The DNA to be expressed is first amplified by the PCR using a 5'-primer that incorporates an area homologous to the 3'-end of the universal promoter. The universal promoter and this **DNA** fragment are mixed and re-amplified in a reaction analogous to splicing by overlap extension, generating a recombinant **DNA template** that can be transcribed and translated in vitro without further processing. Unlike standard methods for in vitro transcription and translation, E-PCR is not dependent upon specialized transcription vectors, cloning, plasmid isolation and purification, or restriction enzyme sites. This approach has been used to synthesize and examine the biological activity of malaria proteins that are vaccine candidates for Plasmodium falciparum. E-**PCR** represents a significant improvement over current in vitro **expression** systems, most notably in its time savings, versatility of gene **expression** and its compatibility with rapid **PCR** -based site-directed mutagenesis procedures.

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